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Chronic Toxicity of Dursban ´Chlorpyrifos´ to the Fathead Minnow ´Pimephales promelas´ and the Resultant Acetylcholinesterase Inhibition

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Fathead minnows (Pimephales promelas) were exposed to Dursban during a chronic toxicity test for 200 days including a reproductive period of their life cycle. The fish concentrated Dursban approximately 1700 times. Survival of first-generation fish was adversely affected at 2.68 µg/liter within 60 days. A significant increase in deformities occurred at 2.68 µg/liter within 30 days. Growth was significantly reduced at 2.68 µg/liter within 30 days and at 1.21 µg/liter by 60 days. Maturation of the first-generation fish was reduced at all Dursban exposures and reproduction was significantly reduced at 0.63 µg/liter and above. Growth and estimated biomass of 30-day-old second-generation fish were significantly reduced at 0.12 µg/liter, the lowest concentration tested. Brain acetylcholinesterase (AChE) activity was significantly inhibited at 0.27 µg/liter and above. AChE inhibition ranged from near 10% in fish exposed to 0.12 µg/liter to 89% for those exposed at 2.68 µg/liter. Inhibition results are compared to other results demonstrated during the chronic study. The use of exposure units (exposure concentration x exposure duration) is discussed as a tool for determining the effects of organophosphate pesticides on the environment.

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Chronic Toxicity of Dursban (Chlorpyrifos) to the Fathead Minnow (*Pimephales promelas*) and the Resultant Acetylcholinesterase Inhibition

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Fathead minnows (*Pimephales promelas*) were exposed to Dursban during a chronic toxicity test for 200 days including a reproductive period of their life cycle. The fish concentrated Dursban approximately 1700 times. Survival of first-generation fish was adversely affected at 2.68 μg/liter within 60 days. A significant increase in deformities occurred at 2.68 μg/liter within 30 days. Growth was significantly reduced at 2.68 μg/liter within 30 days and at 1.21 μg/liter by 60 days. Maturation of the first-generation fish was reduced at all Dursban exposures and reproduction was significantly reduced at 0.63 μg/liter and above. Growth and estimated biomass of 30-day-old second-generation fish were significantly reduced at 0.12 μg/liter, the lowest concentration tested. Brain acetylcholinesterase (AChE) activity was significantly inhibited at 0.27 μg/liter and above. AChE inhibition ranged from near 10% in fish exposed at 0.12 μg/liter to 89% for those exposed at 2.68 μg/liter. Inhibition results are compared to other results demonstrated during the chronic study. The use of exposure units (exposure concentration × exposure duration) is discussed as a tool for determining the effects of organophosphate pesticides on the environment.

INTRODUCTION

Dursban[1] (chlorpyrifos) is an organophosphorous pesticide that is currently registered, or has tolerances pending, for agriculture crops and livestock, ornamental plants, turf, household pests, and mosquito control. The most obvious threat to the aquatic environment is from its use as a mosquito larvicide; however, effects on fish and aquatic invertebrates can also result because of runoff from certain terrestrial uses. Many aquatic species are extremely sensitive to Dursban (Marshall and Roberts, 1978). Increased potential for aquatic environmental damage is possible through the encapsulation of this pesticide in a formulation which permits slow controlled release over an extended period of time. Use of controlled-release Dursban has been demonstrated to control mosquitos for 52 weeks (Evans, 1977). Adverse effects on nontarget aquatic organisms have been observed at water concentrations similar to those that can be expected to occur in the environment (Brown *et al.*, 1976; Thirugnanam and Forgash, 1977; Jarvinen and Tanner, 1982). There is no known published Dursban chronic test data on nontarget organisms. Jarvinen and Tanner (1982) conducted a 32-day embryo–larval fathead minnow (*Pimephales promelas*) study on both the technical grade and encapsulated formulation and observed significant growth effects at approximately 3 μg/liter. Nonquantifiable behavioral changes, however, were observed at lower concentrations. Therefore, it was decided that a full chronic study could perhaps demonstrate adverse effects at lower concentrations.

[1] Trademark of the Dow Chemical Company. Mention of trade names or commercial products does not constitute endorsement by the EPA.

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Acetylcholinesterase (AChE) inhibition can result from the use of organophosphates such as controlled-release Dursban formulations or repeated applications of short-lived formulations. Weiss (1958, 1961) demonstrated that cholinesterase inhibition by successive exposure to organophosphates is additive. Thirugnanam and Forgash (1977) demonstrated cumulative acetylcholinesterase inhibition with time for the mummichog (*Fundulus heteroclitus*) at Dursban water concentrations as low as 1 μg/liter. Fish brain AChE measurements have been recommended as an indicator of organophosphate pollution in the natural environment (Coppage *et al.*, 1975; Coppage and Braidech, 1976).

Objectives of this study were to determine the chronic effects of Dursban on the fathead minnow through a complete life cycle and to compare acetylcholinesterase inhibition to effects on survival, growth, and reproduction. In addition, this study provided baseline laboratory data for direct comparison to field data generated during Dursban exposure to biota in outdoor experimental channels and thereby aided in the comparison of laboratory to field data.

MATERIALS AND METHODS

Physical Conditions

The study was conducted at the Environmental Research Laboratory–Duluth, Duluth, Minnesota.

Test water was sand-filtered Lake Superior water sterilized with ultraviolet light and warmed to approximately 25°C by a coiled stainless-steel heat exchanger located in a stainless-steel headbox.

The basic design of the chronic exposure followed the recommended procedures described by Benoit (1981). A proportional diluter (Mount and Brungs, 1967) was used to deliver duplicates of a control and five toxicant concentrations with a toxicant dilution fator of 0.5. The adult and larval exposure chambers and flow rates to each were the same as those described by Jarvinen *et al.* (1977).

The flow of water to the test chambers was adjusted to maintain dissolved oxygen levels at >75% saturation and to provide a 99% replacement time of the test water in each test chamber within 10 hr as determined from Sprague (1969).

The toxicant was prepared in a saturator system similar to that described by Jarvinen and Tanner (1982) and was pumped to the toxicant mixing chamber of the proportional diluter by a FMI pump (Fluid Metering, Inc., Oyster Bay, N.Y.) calibrated to dispense the desired toxicant flow.

Fish chambers were siphoned daily 1 to 2 hr after feeding to remove leftover food and were brushed and siphoned when algal growth became noticeable.

A constant 16-hr photoperiod was maintained; daylight light intensity between individual fish chambers varied from 28 to 41 L at the water surface.

Biological Conditions

In general, the procedures used followed those in the User's Guide for Conducting Life-Cycle Chronic Toxicity Tests with Fathead Minnows (Benoit, 1981). Fathead minnow larvae less than 24 hr old were obtained from the Environmental Research Laboratory—Duluth Fish Culture Unit. At the start of the study 35 newly hatched larvae were randomly placed in each of the 24 larval growth chambers (two larval

chambers in each large chamber). The fish were fed recently hatched brine shrimp nauplii three times daily on week days and twice on weekends for the first 30 days; thereafter, they were fed frozen adult brine shrimp twice daily until termination of the experiment. The fish were photographed at 30 and 60 days by the method of Martin (1967) as modified by McKim and Benoit (1971) for growth, survival, and deformity determinations. After 60 days the fish in the duplicate larval chambers within each main chamber were combined and randomly thinned to 25. These fish were then released to the adult section of the exposure chamber. Excess fish were killed in ice water, weighed, and frozen at -20°C until analyzed for tissue residues on a whole-body wet-weight basis. At 136 days the fish in each chamber were separated according to sex as described by Benoit (1981) and four spawning pairs selected. Stainless-steel dividers were place in the exposure chamber as were halved cement spawning tiles, and one spawning pair of fish was placed in each of the four compartments. Excess fish were weighed, measured, and frozen at -20°C. At 200 days all remaining fish were weighed and measured.

Ten fish were sampled from each larval chamber for acetylcholinesterase determinations at 60 days and from each adult chamber at 136 days. All remaining fish were sampled when the study was terminated at 200 days. Fish sampled for acetylcholinesterase inhibition were killed in acetone, their skull caps were cut away, and the brains were removed. The brains were placed in aluminum weighing pans that were prerinsed with acetone and weighed to the nearest milligram. Samples were kept on dry ice and immediately brought to the chemistry laboratory where they were processed.

Embryo hatchability studies were conducted on groups of 50 eggs. Larvae at hatch were transfered in groups of 25 to larval chambers for second-generation 30-day growth, survival, and deformity determinations. When second-generation larvae were selected for placement in growth chambers, care was taken so that no two groups of larvae were from the same parent fish. At 30 days, the second-generation fish were killed in ice water and weighed and measured to the nearest milligram and millimeter.

Chemical Conditions

Water temperatures were maintained between 24.3 and 25.9°C and were checked daily in all test chambers. Routine water chemistries were determined weekly by the methods described by the American Public Health Association *et al.* (1980). Dissolved oxygen levels ranged from 6.3 to 8.7 mg/liter. Mean total hardness, acidity, and alkalinity were 43.1, 2.6, and 41.9 mg/liter, respectively; pH was between 7.2 and 7.7.

Dursban (chlorpyrifos; *O,O*-diethyl-*O*-(3,5,6-trichloro-2-pyridyl)phosphorothioate) solution was prepared with Dursban[2] 10 CR obtained from the Dow Chemical company, Midland, Michigan. The encapsulated slow-release formulation was used because of its ease in handling and because the toxicity of the technical grade (98.7% purity) and the encapsulated formulation are similar (Jarvinen and Tanner, 1982). Toxicant concentrations in the fish chambers were determined weekly. Dursban was extracted from the test water with hexane and analyzed by gas chromatography. In each sample set, analyses were made on a duplicate and a spiked sample of control water. The

[2] Dursban 10 CR has been discontinued as a Dow product for economic reasons (A. J. Watson, Dow Chemical, Minnetonka, Minn., Personal communication).

spiked control water samples were spiked at Dursban concentrations similar to the range of those tested; recovery averaged 101.4 ± 10.6% (n = 57). Dursban water concentrations in the test exposures (duplicate samples combined) are presented in Table 1.

Brain samples for acetylcholinesterase determinations were homogenized in distilled water with a hand grinder (5 mg tissue/1 ml water) and were then sonified for approximately 10 sec while on ice. Four 2-ml aliquots were taken from each sample; two were analyzed and the other two frozen and held in reserve. The aliquots to be analyzed were kept on crushed ice until used. Brain acetylcholinesterase activity was determined by the pH-stat method as described by Coppage (1971) and U.S. EPA (1980) and was expressed as the micromoles of ACh hydrolyzed per milligram wet weight of brain tissue per hour.

Residue samples (60-day-old fish) were extracted in an explosion-proof blender. Anhydrous Na_2SO_4 was added to dry the tissues and ensure complete homogenization. Samples were blended with 100 ml of hexane for 2 min at low speed. The solvent extract was decanted onto an anhydrous Na_2SO_4 column and collected in a beaker. Each sample was extracted three times, and the combined extracts were cleaned up on a Florisil column. Samples were eluted with 100 ml of 6% ethyl ether–petroleum ether as described by the U.S. Department of Health, Education, and Welfare (1971). Samples were concentrated on a steam bath and analysis was completed by gas chromatography.

Statistics

Survival results were analyzed by calculation of the accumulative probability of fish survival over the different sample periods during the toxicity study as described by Jarvinen *et al.* (1976).

All survival, embryo hatchability, normal, and sexually mature fish data were transformed to arcsin $\sqrt{\%}$ (Dixon and Massey, 1957; Steel and Torrie, 1960). One-way analysis of variance (P = 0.05) was applied to all survival, embryo hatchability, normal fish, mature fish, growth, reproduction, and biomass data to determine the pesticide effect. Dunnett's procedure (P = 0.05) (Steel and Torrie, 1960) was used to compare treatment with control means.

TABLE 1

DURSBAN WATER CONCENTRATIONS IN THE EXPOSURE CHAMBERS

Nominal dursban water concentration (μg/l)	Measured concentration (μg/l)	
	N^a	Mean ± SD
3.00	57	2.68 ± 0.26
1.50	57	1.21 ± 0.15
0.75	114	0.63 ± 0.09
0.37	58	0.27 ± 0.06
0.18	57	0.12 ± 0.02
Control	57	0.00 ± 0.00

[a] Number of samples.

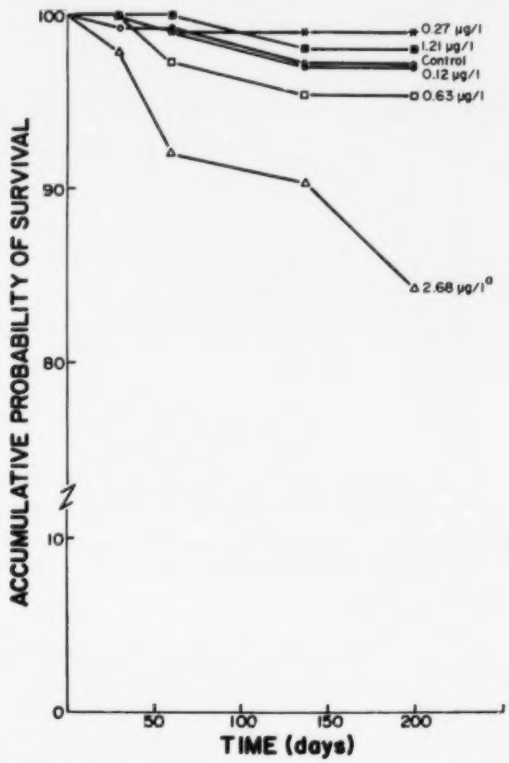

FIG. 1. Accumulative probability of survival for fish exposed to Dursban for 200 days ([a] significantly different from the control).

RESULTS

Survival of the test fish was significantly reduced at 2.68 µg/liter (Fig. 1). Significant reduction, however, did not occur until between 30 and 60 days.

Growth (length) of the first-generation fish was adversely affected at 2.68 µg/liter within 30 days and at 1.21 µg/liter by 60 days (Table 2). Deformities occurred in

TABLE 2

GROWTH (LENGTH)[a] AT VARIOUS TIME INTERVALS FOR FATHEAD MINNOWS CHRONICALLY EXPOSED TO DURSBAN

N	Exposure (days)	Mean measured water concentration (µg/l)					
		0.00	0.12	0.27	0.63	1.21	2.68
140	30	26.5 ± 2.8	26.4 ± 3.5	26.7 ± 2.8	26.4 ± 3.5	26.1 ± 2.5	22.4 ± 3.6[b]
100	60	33.6 ± 3.6	34.0 ± 3.7	34.5 ± 3.5	33.3 ± 4.1	32.2 ± 4.0[b]	28.0 ± 4.9[b]
50	136	49.7 ± 4.9	49.9 ± 8.8	52.4 ± 7.4	48.7 ± 5.6	48.3 ± 5.8	40.6 ± 5.6[b]
16	200	62.6 ± 7.9	62.5 ± 9.6	63.0 ± 11.0	61.5 ± 8.5	58.8 ± 9.0	57.2 ± 8.4

[a] Values expressed as mm ± SD. Each fish entered individually; duplicate chambers combined.
[b] Significantly different from the control.

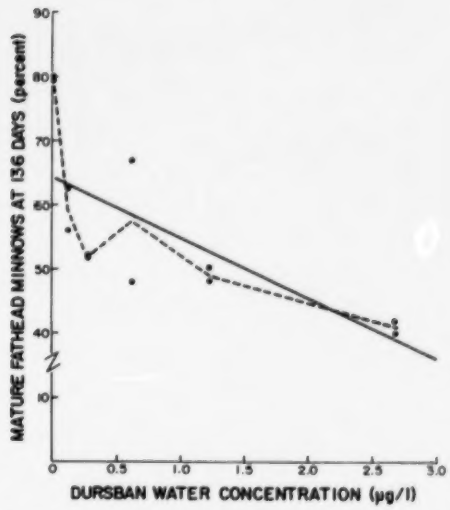

FIG. 2. Maturation of fathead minnows exposed to Dursban. Straight line is the linear regression; correlation coefficient (r) = 0.71. Each dot represents the percentage mature fish (n = 24 to 25) in an individual test chamber.

fish exposed at 2.68 µg/liter during the first 136 days of the study and were manifested within the first 30 days exposure. The typical deformity can be described as a constriction of the body behind the opercular region, fattening of the body behind the constriction, and a shortening of the caudal peduncle. However, scoliosis was also observed in male fish at spawning time. Male fish showing scoliosis consisted of one each at 0.12 and 0.63 µg/liter and two each at 1.21 and 2.68 µg/liter.

Dursban affected the maturation process of the exposed fish (Fig. 2). A significant reduction in the number of sexually mature fathead minnows was observed at all test exposures when compared to controls at 136 days. There were 25% fewer mature fish among those exposed at 0.12 µg/liter and 48% fewer at 2.68 µg/liter.

Tissue residues determined in 60-day-old fathead minnows indicated a mean Dursban bioconcentration factor (concentration in fish, whole body wet wt (µg/g)/concentration in the water (µg/liter)) of 1673 ± 423 (n = 10).

Fathead minnow reproduction was significantly reduced by exposure to Dursban (Table 3). The mean number of spawns per spawning pair of fish was lower at all Dursban exposures than in the controls. A significant reduction was observed at 2.68 µg/liter. The mean number of eggs produced per spawn per spawning pair of fish was also reduced at all test exposures with significant reductions occurring at 0.63 and 2.68 µg/liter. The total number of eggs produced was 44% lower at 0.12 and 0.27 µg/liter approximately 60% lower at 0.63 and 1.21 µg/liter and 89% lower at 2.68 µg/liter when compared to the controls. Total egg production at Dursban exposures of 0.63 µg/liter and higher was significantly lower than in the controls. Embryo hatchability was significantly reduced at 2.68 µg/liter.

Second-generation fathead minnows exposed for 30 days after hatch were more sensitive to Dursban than were parent stock (Table 4). Significant growth (weight) reduction occurred at all test exposures except 0.63 µg/liter. Thirty-day survival of

TABLE 3

EFFECT OF CHRONIC DURSBAN EXPOSURE ON FATHEAD MINNOW REPRODUCTION

	Mean measured water concentration (μg/l)					
	0.00	0.12	0.27	0.63	1.21	2.68
Mean spawns per spawning pair of fish (n = 8)	8.0 ± 1.4[a]	7.0 ± 2.7	6.0 ± 4.0	5.4 ± 3.6	4.3 ± 3.6	2.3 ± 4.0[b]
Mean eggs per spawn per spawning pair of fish (n = 8)	150.1 ± 57.2	95.0 ± 50.3	102.6 ± 60.7	83.3 ± 57.0[b]	108.5 ± 46.2	23.6 ± 37.6[b]
Total egg production (n = 2)	5003 ± 1126	2770 ± 1261	2797 ± 1541	1803 ± 106[b]	2222 ± 45[b]	564 ± 605[b]
Embryo hatchability (%) (n = 7)	95.4 ± 1.7	91.7 ± 3.7	92.5 ± 3.7 (n = 6)	88.9 ± 7.3	89.7 ± 5.5	82.8 ± 16.7[b] (n = 2)

[a] ±SD.
[b] Significantly different from the control.

fish exposed at 2.68 μg/liter was about half that in the controls, but this reduction was not significant because of high variability between duplicates. Two larval chambers were set up; however, larvae from one parent stock had 100% survival whereas the other larval group, from different parent stock, had only 8% survival. From the group that had 100% survival, 52% were deformed. In the other larval group all of the surviving fish were deformed. Some deformities were also observed among the two groups of larvae exposed at 1.21 μg/liter. Percentages for these deformities were 8% in one group and 2% in the other. As for the parent fish, however, only exposure at 2.68 μg/liter caused a significant increase in deformities. One group of larvae from parent fish exposed at 2.68 μg/liter was transferred at hatch to control water for 30 days. This group of larvae had 100% survival and no deformities, and growth was similar to that for second-generation control fish.

To determine the possible ecological significance of our results for comparison to field situations, we calculated the biomass of second-generation fish recruitable to the population or those available as food for predators at 30 days of age. Assuming that

TABLE 4

CHRONIC DURSBAN EFFECTS ON SURVIVAL, NORMAL APPEARANCE, AND GROWTH (WEIGHT AND LENGTH) IN SECOND-GENERATION FATHEAD MINNOWS EXPOSED FOR 30 DAYS AFTER HATCH

	Mean measured water concentration (μg/l)					
	0.00	0.12	0.27	0.63	1.21	2.68
Survival (%) (n = 2)	100.0 ± 0.0[a]	95.0 ± 7.1	99.0 ± 1.4	94.0 ± 0.0	98.0 ± 0.0	54.0 ± 65.1
Normal (%) (n = 2)	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	94.0 ± 2.8	24.0 ± 33.9[b]
Growth						
Weight (mg)	191.7 ± 49.8	174.9 ± 31.7[b]	170.0 ± 35.9[b]	180.8 ± 33.8	169.7 ± 43.5[b]	145.5 ± 35.1[b]
Length (mm)	29.1 ± 2.5 (n = 100)	28.7 ± 1.6[c] (n = 97)	28.3 ± 1.8[b] (n = 99)	28.9 ± 1.8 (n = 94)	27.7 ± 2.6[b] (n = 98)	24.4 ± 2.1[b] (n = 27)

[a] ±SD.
[b] Significantly different from the control.
[c] 0.1 mm less would make this value significantly different from the control.

TABLE 5

ESTIMATED VIABLE BIOMASS RECRUITABLE TO THE POPULATION AT 30 DAYS FROM SECOND-GENERATION FATHEAD MINNOWS FOLLOWING CHRONIC EXPOSURE TO DURSBAN

	Mean measured water concentration (μg/l)					
	0.00	0.12	0.27	0.63	1.21	2.68
Viable biomass (±SD)	902.8 ± 99.2	420.3 ± 188.2[a]	435.6 ± 241.5[a]	273.5 ± 9.8[a]	332.1 ± 2.8[a]	70.5 ± 98.6[a]

[a] Significantly different from the control.

our fish are a population with normal distribution, then $B = \text{TEP} \times H \times S \times G$, where B is biomass in grams, TEP is the total egg production, H is embryo hatchability, S is survival, and G is mean weight in grams. Our results (Table 5) demonstrate a significant reduction in biomass at all test exposures when compared to the controls. The reduction in biomass at 0.12 and 0.27 μg/liter is 52%; at 0.63 and 1.21 μg/liter, 60 to 70%; and at 2.68 μg/liter, 90%.

Brain AChE activity was significantly inhibited at 0.27 μg/liter and above by 60 days of exposure (Fig. 3). Fish exposed at 0.12 μg/liter did not exhibit AChE inhibition until spawning time (136 days), when about a 10% decrease in activity was observed. However, this decrease was not significantly different from the controls. It appears that AChE inhibition was at an equilibrium within 60 days since values observed at the three sampling times are within 2 standard deviations of each other. Percentage inhibition of AChE in the fathead minnows at the various sampling intervals ranged from 0 to 10% at 0.12 μg/liter, 21 to 41% at 0.27 μg/liter, 59% at 0.63 μg/liter, 65 to 80% at 1.21 μg/liter, and 77 to 89% at 2.68 μg/liter.

DISCUSSION

Growth effects in our study occurred within 30 days at a Dursban exposure (2.68 μg/liter) comparable to that demonstrated by Jarvinen and Tanner (1982), who exposed

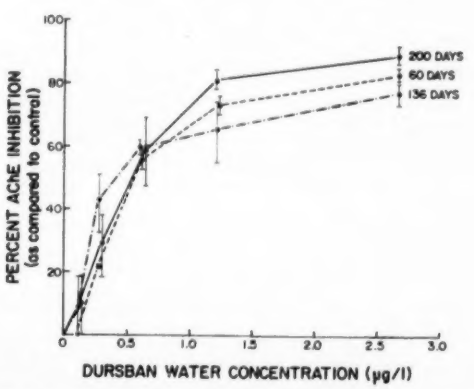

FIG. 3. Percentage brain acetylcholinesterase inhibition in fathead minnows exposed to various Dursban water concentrations for up to 200 days. Vertical bars indicate standard deviation; inhibition was significantly increased at all exposure concentrations except 0.12 μg/liter at all time intervals.

fathead minnows to technical grade and encapsulated Dursban during a 32-day embryo–larval study. In their study a "no effect" concentration based upon growth was between 2.2 and 3.2 μg/liter. At 60 days we observed a significant growth reduction in fish exposed at 1.21 μg/liter and a significant reduction in survival for fish exposed at 2.68 μg/liter. This would indicate a cumulative Dursban effect. Jarvinen and Tanner (1982) demonstrated a significant reduction in fish survival at 4.8 μg/liter within 30 days of exposure. Lack of significant growth differences for fish exposed at 1.21 μg/liter at the 136- and 200-day and at 2.68 μg/liter at the 200-day sample times in our study was because of decreased sample size caused by thinning and sampling the test fish. This resulted in fewer degrees of freedom during statistical analysis. This explanation is likely because the growth differences between the controls and fish exposed at 1.21 and 2.68 μg/liter remained similar or were increased in the 136- and 200-day samples.

In our study the mean eggs per spawn per spawning pair of fish was greater for fish exposed at 1.21 μg/liter than for those exposed at lower concentrations. This is perhaps related to normal biological variability since total egg production at 1.21 μg/liter was significantly lower than the controls and was below that observed at 0.12 and 0.27 μg/liter. Fathead minnow variability also most likely accounts for the slightly better growth of second-generation larvae exposed at 0.63 μg/liter than for those exposed at lower concentrations. Variable response was observed among fish exposed at 2.68 μg/liter, where only two of the eight spawning pairs of fish spawned adequately to provide embryos for hatchability studies. Only one of these two pairs, however, appeared highly resistant to Dursban and accounted for 85% of all the egg production at that exposure. The male fish of that pair was healthier in appearance and was more aggressive than other male fish at the same test exposure. Variable responses to a lesser degree were also observed among fish at lower Dursban exposures. Variable fish response to organophosphate pesticides during chronic exposure has also been observed for fathead minnows exposed to diazinon (Allison and Hermanutz, 1977), for flagfish (*Jordanella floridae*) also exposed to diazinon (Allison, 1977), and for bluegills (*Lepomis macrochirus*) exposed to malathion (Eaton, 1970).

An acute–chronic ratio of 1000 can be calculated by using the 96-hr acute value (120 μg/liter) determined by Jarvinen and Tanner (1982) and the lowest chronic growth effect concentration (0.12 μg/liter) demonstrated for second-generation fish in our study. This ratio is similar to one demonstrated by Allison and Hermanutz (1977) for fathead minnows exposed to the organophosphate diazinon. If the 96-hr fathead acute value (120 μg/liter) observed by Jarvinen and Tanner (1982) is compared with acute values for some other species, it appears that fathead minnows are not as sensitive. The 96-hr LC_{50} for bluegills is 3.6 μg/liter (Macek *et al.*, 1972) and that for the striped bass (*Morone saxatilis*) 0.58 μg/liter (Korn and Earnest, 1974). Therefore, it is possible that these more acutely sensitive species would be chronically affected at Dursban exposures lower than those observed with fathead minnows in the present study. Dursban water concentrations at which we observed chronic fathead minnow effects have been demonstrated to occur in the natural environment. Evans (1977) observed that use of controlled-release Dursban at a dosage of 0.5 to 1.0 mg/liter active ingredient could control mosquitos for up to 52 weeks. Mean water concentrations reported for these two dosages were 0.19 and 0.52 μg/liter, respectively.

The greater sensitivity of second-generation fish observed in our study indicates that there is either a genetic effect in the parent fish or a transfer of Dursban through

the parent fish to their progeny. A possible mechanism would be transfer of residues to the embryo. Dursban was shown in our study to bioconcentrate approximately 1700 times in 60-day-old fish. Marshall and Roberts (1978) summarized the concentration factors determined for Dursban from eight studies on 13 fish species exposed under various experimental conditions. The geometric mean concentration factors calculated for these 13 species range from 21 to 2399.

Since we demonstrated growth and biomass effects on second-generation fish at our lowest tested concentration we cannot state a "no effect" water concentration for Dursban and fathead minnows. We can say only that a "no effect" level is less than 0.12 μg/liter. Comparison of this value to the "no effect" concentration demonstrated by Jarvinen and Tanner (1982) for fathead minnows in an embryo–larval study indicates that our full chronic study is greater than 20 times more sensitive. This difference, however, is not large if the data are compared by exposure units (exposure concentration × exposure duration) as described by Allison (1977). Thus, fish survival was significantly affected at 154 to 182 Dursban exposure units as calculated from Jarvinen and Tanner (1982) and at 161 exposure units in our study. Growth was significantly affected at 96 exposure units (Jarvinen and Tanner, 1982) and at 86.7 and 72.6 exposure units at 30 and 60 days, respectively, in our study. Significant reproductive effects in our study occurred between 136 and 200 days of exposure at 0.63 μg/liter, which would be 85.7 to 126 exposure units. The 96-hr LC_{50} values (Jarvinen and Tanner, 1982) for technical grade and encapsulated Dursban converted to exposure units would be 480 to 560. In our study about 50% survival of second-generation fish occurred at an exposure of 2.68 μg/liter at 200 days (total first- and second-generation exposure), or 536 exposure units. Thus, the exposure unit concept appears to hold promise (at least for this fish and chemical) as a means of estimating certain effects at untested exposure intervals. The only exception would be that we observed significant growth effects in second-generation fish at 0.12 μg/liter (24 exposure units), a factor three to four times lower than the number of exposure units which caused growth effects in our first-generation fish and in those from the embryo–larval study of Jarvinen and Tanner (1982).

A comparison of brain acetylcholinesterase inhibition with other results indicated that whenever inhibition occurred in the test fish adverse effects were also observed at some point in the fishes life cycle. When AChE inhibition was near 80% or greater, survival, growth, deformities, and embryo hatchability were significantly affected; at 50% inhibition or greater, significant reproductive effects occurred; and at 10 to 40% inhibition or greater, fish maturation and second-generation growth were significantly reduced. This would indicate that measurement of brain AChE levels has the potential for being a useful monitoring tool for determining adverse pollution problems in the environment. Macek *et al.* (1972) demonstrated that bluegills and largemouth bass (*Micropterus salmoides*) exposed to Dursban in experimental ponds exhibited more than 80% inhibition of brain AChE when exposed to water concentrations causing 55 and 46% mortality. Maximum inhibition occurred within 3 to 7 days. In our study the test fish were too small for accurate AChE determinations until 60 days, at which time it appeared that an inhibition equilibrium had been reached. Thirugnanam and Forgash (1977) observed that mummichogs (*Fundulus heteroclitus*) exposed to Dursban during a field exposure had 56 to 100% inhibition with a corresponding mortality of 18.6%. The same authors in an earlier study (Thirugnanam and Forgash, 1975) determined that brain AChE inhibition was proportional to the pesticide con-

centration of temefos and the exposure period for the mummichog and sheepshead minnow (*Cyprinodon variegatus*). The authors stated that visible symptoms of poisoning were apparent only after AChE inhibition reached 80%. Coppage (1977) demonstrated that brain AChE inhibition of survivors was similar (77 to 89%) regardless of compound, species, or period of exposure (4 to 48 hr) when mortality was 40 to 60% for the pinfish (*Lagodon rhomboides*), sheepshead minnows, and sailfin molly (*Poecilia latipinna*) exposed to five different carbamate pesticides. He stated that it was likely that brain inhibition greater than 70% was related causally to poisoning in acute exposure and indicates that some deaths have occurred or will occur in fish populations exposed to anticholinesterase agents. In our study significant reduction of fish survival occurred within 60 days where the fish showed 77 to 89% brain AChE inhibition. However, mortality of these fish was only 8 to 16% over the entire 200-day study. Eaton (1970) observed a near 50% reduction of brain AChE activity in bluegills exposed to harmful levels of malathion and about 30% inhibition at toxicant concentrations considered safe during a chronic 180-day study. In our study inhibition as low as 10% was related to an unsafe Dursban concentration.

When toxic nonpersistent pesticides are recommended for use in slow-release encapsulated formulations and land-usage patterns indicate a potential for contamination, possible effects on sensitive nontarget aquatic organisms should be determined during life-cycle testing before registration is permitted. Studies should also be conducted to determine whether high-dosage pulses of short duration, as commonly result from the current pesticide application procedures, are capable of causing chronic effects in nontarget aquatic organisms through cumulative action. Use of the exposure unit concept may be a valid approach in predicting certain ecological effects of organophosphate pesticides on the environment.

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